REPORT OF 3rd EXCHANGE on EUROPEAN HAKE AGE READINGS (SAMFISH)

by
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1. Introduction

According to the Workplan of EUContract No. 99/009 (Improving Sampling of Western and Southern European Atlantic Fisheries – SAMFISH) an otolith exchange scheme should be undertaken on hake (*Merluccius merluccius)* for the Northern and Southern Stocks in order to calibrate the age readings.

The exchange was carried out following the recommendations of the EFAN Report 3-2000 on Guidelines and Tools for Age Reading Comparations, which is available in the EFAN home page. In order to check the precision in age reading and bias of the age readers of this species, the background for ageing hake was based on two reports of *Hake Otolith Age Reading Workshops* conducted previously (*1997 and 1999*). They are available in the EFAN home page (*http://www.efan.no*; *Report 6-2000 and Report 7-2000*).

2. Objectives:

- Analysis of the results of the hake otolith exchange in order to check the precision in age reading and bias of the age readers referred to at the last workshop.
- Also new readers become involved in hake age determination and need to get used to the otolith interpretation criteria.

3. Participants:

Reader	Name	Institution	City	Country
Reader 1	M. Saínza	IEO	Vigo	Spain
Reader 2	M. H. Afonso	IPIMAR	Lisbon	Portugal
Reader 3	C. Piñeiro	IEO	Vigo	Spain
Reader 4	J. Labastie	IFREMER	La Rochelle	France
Reader 5	S. Arego	AZTI	Sukarrieta	Spain
Reader 6	S.Warnes	CEFAS	Lowestoft	England
Reader 7	A. Payne	CEFAS	Lowestoft	England
Reader 8	T. Watson	CEFAS	Lowestoft	England

Reader 9	F. Woods	MI	Dublin	Ireland
Reader 10	H. Mc Cormick	MI	Dublin	Ireland
Reader 11	C. Morgado	IPIMAR	Lisbon	Portugal
Reader 12	M. Marín	IEO	Vigo	Spain

4. Material and Methods

A collection of 199 Hake otolith sections from two ICES areas (99 from Sub-area VII and 100 from Divisions VIIIc + IXa) was circulated among interested Institutes during 2001. The exchange scheme started on the 20th of January 2001 and finished in 31st of November (Figure 1). Digitised images from otoliths sections were stored in a CD Rom, which accompanied the exchange collection. Unfortunately the otolith collection was lost when the last institute sent it to the co-ordinator in Vigo but the digitised images still remain in the CD Rom.

The length range of the fish sampled was between 11cm and 83 cm. Information on catch date, sex and total length were available for the readers. The otolith sections were prepared in Vigo (IEO) with the same technique used in the previous exchanges. The length and age distribution sampled is shown in Figure 2.

Before circulating the otolith collection an ageing protocol was provide to all readers (Annex 1). In order to know whether the readers count the same rings, the first three rings and the check ring considered by reader for age estimation, were measured. For Reader 8, only distances of the first annual ring were considered for the analysis since the following rings measurement presented problems with units.

Regarding to the level of experience of the readers involved in the exchange there was differences. On one hand, there were hake readers traditionally involved in hake stock assessment, more over, there were readers who participated in the three hake otolith exchanges and on the other hand, there were new readers in this exchange. So initially, it was decided to split them into 4 groups:

- Readers involved in hake stock assessment: R1, R2, R3, R4, R5
- Most experienced readers: R3, R4
- Readers with some experience: R9, R10, R11, R12
- New readers: R6, R7, R8

The general criteria adopted for ageing is described in the report of the previous exchange (*Hake Otolith Age Reading Workshop*, 1999*; EFAN* 7-2000).

The analysis of the age reading results was done by mean the Excel ad-hoc Workbook "AGE COMPARATIONS. XLS" from A.T.G.W. Eltink from RIVO.

The basic requirement for age reading consistency is the absence of bias among readers and through time. One of the ways to test this absence of bias between two readers or between a reader and the modal age estimated is with a one-sample Wilcoxon signed rank test, which was, chosen in this exchange in order to compare with the previous test applied in the first exchange.

To study the variability in the precision of age determinations between readers an

extensive analysis was conducted to provide more details concerning individual's performances:

1. Exploratory data analysis (EDA)

- Determination of the modal age and of the difference between each readers' age and the modal age. The modal age was calculated based on the results of readers involved in stock assessment: R1, R2, R3, R4 and R5. In case of bi-modality the modal age was estimated from the most experienced readers (R3 and R4), and when the analysis is performed for only these two readers, the bi-modality is solved with the modal age from the readers R1-R5 involved in stock assessment

- Graphical representation by reader for the sample using box-whisker plots (median and interquartil range by reader). The box-whisker plots were used to summarise the observations and are useful in observing and comparing the distribution of the otolith readings by reader.

2. In terms of reproducibility measures:

2.1) Average percent age error (APE), Beamish and Fournier (1981) is an index of reading precision to compare a series of observations. The formula is as follows:

$$APE = \frac{100}{n}\sum_{i=1}^{n}\left(\frac{1}{r}\sum_{j=1}^{r}\frac{\left|x_{ij}-\bar{x}_i\right|}{\bar{x}_i}\right) \quad (1)$$

n = number of otoliths
r = number of readings for each otolith
x_{ij} = the j value of age estimation for the i otolith
$\bar{x}_i$ = average age calculated for the i otolith

2.2) The Mean Coefficient of Variation (CV)

$$CV = \frac{100}{n}\left[\sum_{i=1}^{n}\left(\frac{sd}{\bar{x}_i}\right)\right] \quad (2)$$

sd = the standard deviation for the i otolith

2.3) The index of precision (D) (Chang, 1982):

$$D = \frac{100}{n}\left[\sum_{i=1}^{n}\frac{CV}{r^{0.5}}\right] \quad (3)$$

CV is described in 2.2)

These measurements are more appropriate than the conventional percent of agreement when comparing ages, since those take into account the average year class of fish.

3. In terms of grouping readers with different agreement between them:

- Hierarchical cluster analysis using average linkage (between groups) based on squared Euclidean measure for readers without transforming the data.

- Multiscaling dimension (MSD) to show the multidimensional space based on squared Euclidean measure for readers without the transformation of input data using an ordinal measure scale.

To know if there were differences in the ages assigned for Southern and Northern stock samples, a Kruskal-Wallis non parametric analyses was carried out based on the readings from readers involved in hake stock assessment: R1, R2, R3, R4, R5

5. Results and discussion

The results of the readings and the basic information about otolith collection are summarised in Table 1

In general, the box-whisker plot for all readers shows that the ages attributed are very similar (average = 2.5 years) except readers R7, R8, and R9. The first results pointed out two main groups which it can be related to experience and inexperience readers (Figure 3a):

1/ R1, R2, R3, R4, R5, R6, R10, R11and R12
2/ R7, R8, and R9

The box-whisker plot for first ring distance measured for all readers shows two main groups, the first group include R1, R2, R3, R4, R5, R9, R10 and R12 consisting of readers with some experience and the other group include mainly readers without experience: R6, R7, R8, and R11 (Figure 3b). The graphical representation illustrates that, readers with experience present similar average distances (1.3 mm).

The box-whisker plot for check ring distance measured for all readers pointed out that, with exception of readers R7 and R11, all readers can distinguish clearly this check (Figure 3c). Its average distance to the nucleus seems to be around 1.5 mm., this coincide with the distance reported by Piñeiro and Hunt, 1989. This is a very hopeful result since this check is the reference ring to start the counting.

The box-whisker plot for second and third ring distance measured indicates again that the majority of readers are in agreement and present similar average measurements with exception of R2, R7 and R11 readers. (Figure 4a,b). It should be noted that Reader R2 presents a very consistent pattern in the identification of the rings.

In relation with the location of the first three rings, the graphical indicates that in this

exchange there is the highest agreement among readers obtained until now. Only the lack of experience in hake ageing and the misinterpretation of the ageing criteria are likely causes for any discrepancies observed. Taken into account that this aspect is one of the major difficulties in the age interpretation of this species, the result is an encouraging sign.

Considering the bias plots for all readers together it can be observed that the mean age recorded is very close to the modal age and the deviations increase relatively with age. High bias was observed in readers R7 and R9 which are overestimated, and R8 which is underestimated relative to the modal age. Readers R5, R6 and R12 underestimate ages older than 6. However the results for all readers present less bias than the previous exchanges (Figure 5). A high precision is achieved by readers: R1, R2, R3, R4, R5, R11 and R12

The coefficient of variation (CV%) and percent agreement are plotted against modal age in Figure 6. This figure shows these results from all readers, experienced readers involved in hake ALK's (R1-R5) and from the most experienced readers (R3, R4) to see how these measures change with experience. Firstly, from all readers the average of CV was 40 %. This value is so high mainly due to an important effect of age magnitudes in the calculation of CV's. Especially in this exchange, where there was a big amount of younger ages in the sample (0,1,2). In fact, the CV's are much higher for age 0 and afterwards the CV's decreased very much, keeping around 20 % for ages older than three.. Besides that there are difficulties in the discrimination of the first annual ring among the false rings. The percent agreement decreased as the age increased and the mean value obtained was 58%.

Secondly, from readers involved in hake ALK's, the average of CV was 17% and this value was much higher for age 0, mainly due to the important effect of age magnitudes in the calculation of CV's, already mentioned. For older ages, the CV's decreased considerably to values around 10 %. The average percent agreement was 75 %. Finally from most experience readers, the average of CV was 9 % and the average percent agreement was 84%. As can be seen the level of experience affect considerably to the ageing precision and the level of agreement.

Wilcoxon's test between readers is shown in Table 2. The results clearly emphasize the absence of bias for the first six readers (R1, R2, R3, R4, R5, R6). However bias still remained for the rest of the readers.

A plot of mean length at age (Figure 7) can serve to diagnose individual reader tendencies. Except for readers R7, R8, R9, the mean length at age estimated by reader is similar for the first 6 ages above that age the difference increases.

The results of APE, CV, and D indices in (%) for all readers are: 28, 40 and 12 respectively (Table 3). The fact of having a large number of young fish in the sample affects the results of APE and CV since these indices are very sensitive to differences in younger ages, especially 0 and 1. When the analysis is carried out using only the most experienced readers (R3 and R4) these indices decreased considerably to 6, 9 and 6. These results are much lower than those obtained in the previous exchanges (Figure 8) and indicate that there has been a clear improvement in ageing hake.

The dendogram obtained from the hierarchical cluster analysis point out also the presence of two main groups (Figures 9).

Group 1: R1, R2, R3, R4, R5, R11, and R12
Group 2: R6, R7, R8, R9 and R10

In order to clarify the groups, a plot of coordinates from MSD analysis was carried out (Figures 10). It can be seen in the plot that the first dimension splits the readers into two groups, supporting the results of the hierarchical cluster analysis.

The results of the Kruskal-Wallis analyses shown that no significant differences ($p>0.05$) exist between the ages assigned for the same length in both stocks (North and South).

In summary the exploratory and statistical analysis showed two main groups of readers: experienced and inexperienced. Therefore again it is recognised the importance of experience in hake ageing. The lack of experience in ageing is the most likely reasons for the observed discrepancies.

The significant achievement of this exchange is that for the first time, the ageing method for the first 6 ages has provided high level of agreement and very consistent age estimations, including readers partially experienced. For ages older than 6, the agreement amongst readers diminish.

Despite the improvement of all the readers, the results highlight the difficulties in ageing hake otoliths, mainly older fish, and justify the need for periodical exchange exercises amongst the countries involved in stock assessment, particularly focussed on older ages.

6. Conclusions:

From these results a clear improvement in ageing hake is observed comparing the results with those from previous exchanges it can be seen:

- The highest level of agreement in the location of the first three annual rings. This is the result of the adoption of the ageing criteria established.

- The exploratory and statistical analyses showed that ageing is very consistent and the check ring which is the reference ring to start the counting is very well distinguished for practically all readers.

- The results of APE, CV, and D indices in (%) for all readers are the best in the whole series of exchanges.

- The ageing method for the first 6 ages has provided the highest level of agreement and very consistent age estimations, including the readers partially

experienced. For ages older than 6, it is necessary to undertake studies on life history events such as reproduction process in relation to the pattern ring formation in the otoliths.

- Readers involved in stock assessment had a high level of agreement and precision reaching the mean values of 75 % and 17% respectively. Furthermore, new readers showed an adequate interpretation of the ageing criteria.

- No significant differences exist ($p>0.05$) between otoliths from both stocks (North and South).

7. Recommendations

- Exchanges should be carried out among Institutes on a regular basis, especially for those who supply ALK´s to the assessment Working Groups. Furthermore, a workshop should be convened in the future since some ageing difficulties still remain, mainly for the older ages.

- Statistical tests on age data of each Institute should be carried out before they are combined for ALK´s.

- It is desirable that at least two readers in each Institute should be involved in Hake age determination.

- A training guide with digital images of otolith sections from ages 0 to 6 will be helpful to introduce the readers in the ageing criteria established. Likewise a reference collection to calibrate the experienced readers is recommended.

- It is necessary to validate the age estimation of this species by conducting more studies on the life history events of the fish.

- A secure transportation method is essential for the successful completion of the exchange otolith collection and this is responsibility of all participants.

8. Acknowledgements

This otolith exchange was possible to carried out with the participation of all readers from the six institutes involved and the success of the exchange is due to their efforts. Thank is extended to Valentin Trujillo for his suggestions. Thanks also to Guus Eltink form RIVO for permission to use his analytical software AGE COMPARISONS.XLS.

Table 1.- Results of age estimations by reader

Sample **Hake Otolith age reading: 3rth exchange 2001:**

	Fish	Fish		Landing	IEO	IPIMAR	IEO	IFREMER	AZTI	CEFAS	CEFAS	CEFAS	MI	MI	IPIMAR	IEO
Nº.	N.º	length	Sex	month	R 1	R 2	R 3	R 4	R 5	R 6	R 7	R 8	R 9	R 10	R 11	R 12
	10467	20	2	10	1	1	1	1	1	1	1	1	2	2	1	1
	10003	18	2	9	1	1	1	0	1	1	2	1	2	1	1	1
1	10035	16	3	9	1	0	1	0	1	1	2	1	1	1	0	1
	10122	14	3	9	0	0	0	0	0	1	1	1	0	0	0	0
	10346	12	3	9	0	0	0	0	0	1	1	1	0	0	0	0
	10490	11	3	10	0	0	0	0	0	0	1	0	0	0	0	0
	10576	13	3	10	0	0	0	0	0	1	1	1	0	0	0	0
2	10814	15	3	10	0	0	0	0	1	1	2	1	0	0	0	0
	10612	17	3	10	0	1	0	0	1	1	2	1	1	0	0	0
	10638	19	2	10	1	0	1	0	1	1	2	1	1	0	0	1
	10061	21	2	9	1	1	1	1	2	1	2	1	2	1	1	1
	10030	23	3	9	1	1	1	1	2	1	2	1	2	1	1	1
	693	25	3	9	1	1	2	1	2	2	3	2	3	1	1	2
3	700	27	3	9	2	2	2	2	2	2	3	2	3	1	2	2
	638	29	3	9	2	2	2	2	3	-	4	2	3	2	2	2
	10807	22	2	10	1	1	1	1	1	2	2	2	1	1	1	1
	10759	24	1	10	1	1	1	1	2	3	3	2	2	2	1	1
4	10771	26	1	10	2	1	1	2	2	2	3	2	3	2	1	2
	11023	28	1	10	2	2	2	2	3	4	4	3	3	3	1	2
	10699	30	1	10	2	2	2	2	3	2	4	2	3	2	2	2
	616	31	3	9	2	2	2	2	2	-	4	2	3	4	2	2
	631	33	3	9	3	3	3	2	4	5	5	3	4	4	3	3
5	648	35	3	9	2	2	2	2	3	5	4	3	3	3	2	2
	649	37	3	9	3	3	3	3	4	-	5	3	4	4	3	3
	764	39	3	9	3	4	3	3	4	-	5	2	5	7	4	3
	10843	32	1	10	2	2	2	2	3	4	4	2	3	3	2	2
	10675	34	1	10	3	3	2	2	3	4	4	3	4	2	3	3
6	10671	36	1	10	3	3	3	3	4	-	4	2	3	3	3	3
	10569	38	2	10	3	3	3	3	3	4	5	3	3	2	4	3
	10845	40	1	10	3	3	3	3	4	-	5	3	4	2	3	3
	4212	42	3	9	3	4	4	4	4	6	5	4	4	5	3	3
	691	43	3	9	3	4	4	4	4	7	5	2	4	4	4	3
7	676	44	3	9	3	4	4	4	4	-	5	2	5	4	4	3
	679	46	3	9	4	4	4	4	4	-	6	3	5	5	4	3
	4210	48	3	9	4	5	5	5	5	5	6	3	6	6	5	4
	4209	49	3	9	4	6	4	5	5	6	7	3	9	5	6	4
	745	52	3	9	5	6	5	6	5	6	5	4	5	6	6	5
8	726	53	3	9	5	6	5	5	5	6	6	4	6	5	5	4
	746	54	3	9	5	6	5	6	5	-	6	4	10	6	6	4
	749	56	3	9	6	7	6	6	5	6	7	5	5	6	6	6
	10792	43	2	10	3	4	3	3	4	6	5	3	6	4	3	3
	11138	42	1	10	3	4	3	3	4	5	5	2	5	7	3	3
9	11006	42	1	10	3	3	3	3	4	5	4	2	5	5	3	3
	11117	41	2	10	3	3	3	3	4	5	5	3	4	6	3	3
	10913	41	2	10	4	3	3	3	4	4	5	2	6	6	3	3
	10715	60	2	10	5	7	5	6	6	8	10	4	7	6	6	6
	10991	52	1	10	5	5	5	5	5	7	8	3	7	5	5	5
10	4227	48	3	11	4	5	5	4	4	5	6	3	5	4	4	4
	4237	46	3	11	6	6	6	5	4	4	6	3	6	6	5	4
	10898	43	2	10	5	5	4	4	4	4	6	3	5	5	4	4
	123	11	3	1	1	1	1	1	1	1	1	1	0	0	1	1
	135	13	3	2	1	1	1	1	1	1	1	1	0	0	1	1
11	127	15	3	1	1	1	1	1	1	1	1	1	1	1	1	1
	145	17	3	2	1	1	1	1	1	1	2	1	1	1	1	1
	124	19	3	1	1	1	1	1	1	1	2	1	1	1	1	1
	134	12	3	2	1	1	1	1	1	1	1	1	0	0	1	1
	974	14	3	5	1	1	1	1	1	1	1	1	0	0	1	1
12	971	16	3	5	1	1	1	1	1	1	1	1	1	1	1	1
	1010	18	3	5	1	1	1	1	1	1	2	1	1	1	1	1
	983	20	3	5	1	1	1	1	1	1	2	1	1	1	1	1
	528	21	3	3	2	1	2	1	2	2	2	3	2	2	1	2
	144	23	3	2	2	1	2	1	2	2	3	2	2	2	1	2
13	515	25	3	3	2	2	2	2	3	2	3	2	3	2	2	2
	136	27	3	2	3	2	2	2	3	2	4	3	3	3	2	3
	140	29	3	2	3	2	3	2	3	2	4	3	4	4	2	2
	973	22	3	5	2	1	2	1	2	2	3	2	2	2	1	2
	912	24	3	6	2	1	2	1	2	2	3	2	2	1	2	1
14	929	26	3	6	2	1	2	2	3	-	3	2	2	2	1	2
	910	28	1	6	2	2	2	2	3	3	4	2	3	3	2	2

Table 1.- Results of age estimations by reader (continuation)..

Sample **Hake Otolith age reading: 3rth exchange 2001:**

	Fish	Fish		Landing	IEO	IPIMAR	IEO	IFREMER	AZTI	CEFAS	CEFAS	CEFAS	MI	MI	IPIMAR	IEO
Nº.	N.º	length	Sex	month	R 1	R 2	R 3	R 4	R 5	R 6	R 7	R 8	R 9	R 10	R 11	R 12
14	910	28	1	6	2	2	2	2	3	3	4	2	3	3	2	2
	565	30	1	4	2	2	2	2	3	2	4	2	3	3	2	2
15	18	32	2	1	3	2	3	3	3	2	4	3	6	2	2	3
	15	33	2	1	3	2	3	3	3	2	4	2	7	3	2	3
	10	35	2	1	3	2	3	3	3	2	4	2	4	3	3	3
	27	37	2	1	3	2	3	4	3	3	5	2	5	3	3	3
	16	39	2	1	4	4	4	4	4	4	-	3	4	4	4	4
16	544	40	1	4	3	3	3	4	4	4	5	2	4	3	3	3
	557	38	1	4	4	4	4	4	4	3	5	2	6	5	4	4
	549	36	2	4	4	4	4	3	4	3	4	2	4	5	3	3
	548	34	2	4	3	3	4	3	3	3	4	2	4	6	3	3
	545	31	1	4	3	2	3	3	3	-	3	2	4	4	2	3
17	41	41	1	2	3	3	3	4	4	4	4	3	4	3	4	4
	1	42	2	1	4	5	4	4	4	6	4	3	6	6	5	4
	73	43	1	2	5	4	6	4	4	6	5	3	6	7	4	4
	42	44	2	2	5	4	5	4	4	5	-	4	7	7	4	4
	40	45	1	2	6	4	5	5	5	5	-	4	5	6	5	4
18	17	47	2	1	5	5	6	5	5	4	6	3	5	5	5	4
	2	50	2	1	6	6	6	5	5	4	7	4	6	9	6	5
	4	54	2	1	6	5	6	6	5	4	7	3	7	6	5	5
	34	56	2	2	7	7	6	6	6	-	8	4	12	7	7	5
	100	58	3	3	6	7	6	6	6	5	7	3	8	6	7	6
19	575	45	2	4	5	5	4	4	4	4	5	3	4	5	4	4
	568	44	1	4	5	5	5	5	5	4	6	3	6	6	4	4
	569	43	1	4	5	5	5	5	4	-	5	3	6	6	4	4
	538	42	1	4	5	5	5	5	4	4	5	3	5	6	5	4
	580	41	3	4	4	3	4	4	4	3	5	3	4	5	3	4
20	829	61	2	6	7	7	8	6	6	5	8	5	6	8	7	6
	1109	54	3	6	6	7	6	6	6	5	7	3	8	7	6	5
	1107	53	3	6	6	7	6	6	5	4	7	4	7	6	6	5
	1114	47	3	6	5	5	6	5	4	4	6	4	7	5	5	5
	533	46	2	4	5	5	5	4	4	4	5	3	5	4	5	4
21	783	12	3	8	0	0	0	0	0	0	1	0	0	0	0	0
	781	13	3	9	0	0	0	0	0	0	1	0	0	0	0	0
	804	14	3	9	0	0	0	0	0	0	2	0	0	0	0	0
	779	15	3	8	0	0	0	0	1	0	2	0	0	0	0	0
	780	16	3	8	1	1	0	0	1	0	2	0	0	0	0	1
22	793	17	3	9	1	1	1	0	1	0	3	1	1	1	0	1
	587	18	2	7	1	1	1	1	1	0	3	1	1	1	0	1
	582	19	2	7	1	1	1	1	1	0	3	1	1	1	1	1
	570	20	2	7	1	1	1	1	1	0	3	1	1	1	1	1
	576	21	2	7	1	1	1	1	1	1	3	1	2	1	1	1
23	562	22	1	7	1	1	1	1	1	1	4	1	2	2	1	1
	551	23	1	7	1	1	1	1	1	1	3	2	2	2	1	1
	564	24	1	7	1	1	1	2	2	1	4	2	3	3	1	1
	552	25	1	7	1	1	2	2	2	1	3	1	3	2	1	1
	566	26	1	7	1	1	2	2	2	1	4	3	2	2	1	1
24	826	31	2	11	2	2	2	2	3	2	4	2	3	3	2	2
	559	30	2	7	3	2	3	2	3	2	5	2	4	3	2	2
	583	29	2	7	2	2	2	2	2	2	5	2	3	3	2	2
	557	28	1	7	2	2	2	2	2	2	4	1	3	2	2	2
	563	27	2	7	2	2	2	2	3	1	5	2	3	3	2	2
25	784	32	3	8	2	2	2	2	3	2	5	3	3	3	2	2
	796	33	3	9	2	2	3	2	3	3	4	2	3	3	2	2
	549	34	3	7	2	2	3	3	3	2	5	2	3	3	2	2
	828	35	3	11	3	3	2	2	3	2	5	3	4	3	3	2
	814	36	3	11	3	2	3	2	3	2	5	2	3	4	2	2
26	609	37	2	7	3	2	3	3	3	2	5	3	3	3	2	2
	543	38	3	7	3	3	3	3	3	3	5	2	3	4	3	2
	535	39	3	7	3	3	3	3	3	3	6	4	4	4	3	3
	529	40	3	7	3	3	3	4	4	3	5	4	4	4	3	3
	920	41	3	11	3	4	3	3	4	4	6	3	3	3	4	3
27	518	46	3	7	4	4	4	5	4	3	6	5	4	4	4	5
	919	45	3	11	4	4	4	4	4	4	6	4	4	5	4	3
	910	44	3	11	4	3	4	3	4	3	5	5	3	5	3	3
	909	43	3	11	4	3	4	3	4	4	6	5	6	6	3	3
	540	42	3	7	3	3	4	**4**	4	3	5	4	5	5	3	3

Table 1.- Results of age estimations by reader (continuation)..

Sample **Hake Otolith age reading: 3rth exchange 2001:**

	Fish	Fish		Landing	IEO	IPIMAR	IEO	IFREMER	AZTI	CEFAS	CEFAS	CEFAS	MI	MI	IPIMAR	IEO
Nº.	N.º	length	Sex	month	R 1	R 2	R 3	R 4	R 5	R 6	R 7	R 8	R 9	R 10	R 11	R 12
	521	47	3	7	5	4	5	4	5	4	7	3	6	6	4	4
	860	48	3	11	4	4	4	4	4	4	6	3	6	5	4	4
28	864	49	3	11	6	6	5	4	5	4	6	3	5	6	5	5
	857	50	3	11	5	6	5	5	5	4	8	4	6	7	6	5
	859	55	3	11	7	7	6	5	6	4	8	2	7	8	7	5
	575	62	2	7	6	8	6	6	6	5	10	4	6	7	8	6
	502	62	2	7	7	8	7	7	6	6	9	5	6	7	8	7
29	602	61	2	7	7	8	8	7	6	5	9	5	7	7	8	6
	878	59	3	11	6	6	6	6	5	4	8	4	8	8	6	6
	881	58	3	11	8	7	7	6	6	4	9	4	7	7	6	6
	493	83	3	7	9	8	9	11	9	7	14	7	8	8	9	9
	496	76	2	7	8	8	7	10	7	8	13	6	7	9	8	7
30	498	72	2	7	8	8	9	9	7	6	12	5	10	8	8	7
	501	64	2	7	8	8	7	7	6	5	10	6	7	8	8	6
	49	11	3	1	1	1	1	1	1	1	1	1	0	0	1	1
	55	12	3	1	1	1	1	1	1	1	1	1	0	0	1	1
31	54	14	3	1	1	1	1	1	1	1	2	1	0	1	1	1
	53	15	3	1	1	1	1	1	1	1	2	1	1	1	1	1
	785	16	3	3	1	1	1	1	1	1	2	1	0	0	1	1
	52	17	3	1	1	1	1	1	1	1	2	2	1	1	1	1
	776	18	3	3	1	1	1	1	1	1	2	2	1	1	1	1
32	50	19	3	1	1	1	1	1	1	1	2	2	1	1	1	2
	51	20	3	1	1	1	1	1	1	2	2	2	2	2	1	2
	795	20	3	3	1	1	1	1	1	1	2	2	2	2	1	2
	794	21	3	3	1	1	1	1	1	1	3	2	2	2	1	2
	790	22	3	3	1	1	2	1	1	1	3	2	2	2	1	2
33	57	23	3	1	1	1	2	2	2	1	3	2	2	2	1	2
	58	24	3	1	2	1	2	2	2	2	3	2	2	2	1	2
	61	25	3	1	2	1	2	2	2	1	3	2	2	2	1	2
	62	26	3	1	2	1	2	2	2	2	3	2	3	2	1	2
	64	27	3	1	2	2	2	2	3	2	4	2	3	3	2	2
34	67	28	3	1	2	1	2	3	3	2	4	3	4	2	2	2
	454	29	3	2	2	1	2	3	3	2	4	2	3	3	2	2
	489	30	3	3	3	2	3	3	3	2	4	2	3	3	2	3
	68	31	3	1	3	2	3	3	3	3	4	2	3	3	2	3
	69	32	3	1	3	2	3	3	3	2	5	3	3	2	2	2
35	450	33	3	2	3	2	3	3	3	3	4	3	3	2	2	2
	451	34	3	2	3	2	3	3	3	3	5	3	3	2	2	3
	456	35	3	2	3	2	4	3	4	3	-	2	5	3	3	3
	460	36	3	2	3	2	3	3	3	3	4	2	3	2	2	3
	463	37	3	3	3	3	3	4	4	4	5	2	4	2	3	3
36	486	38	3	3	3	3	3	4	4	3	5	3	4	2	3	3
	474	39	3	3	4	3	4	4	4	4	5	3	6	3	3	3
	465	40	3	3	4	3	4	4	4	4	5	2	5	3	3	4
	468	41	3	3	4	4	4	4	5	5	6	3	6	4	4	4
	461	42	3	3	4	3	4	4	4	5	5	2	5	5	4	3
37	473	43	3	3	4	4	4	4	4	4	-	2	5	5	4	4
	462	44	3	3	5	5	5	5	5	5	6	4	5	4	4	5
	475	45	3	3	6	5	7	5	5	-	-	-	-	-	4	4
	504	46	3	3	5	4	5	5	5	4	6	2	6	6	5	5
	464	47	3	3	5	4	5	5	5	5	-	4	6	5	5	5
38	497	48	3	3	4	5	5	6	5	4	7	2	8	5	6	5
	632	49	3	3	5	6	5	5	5	4	6	3	7	5	6	5
	507	50	3	1	5	6	5	6	5	4	7	3	7	6	6	5
	1036	51	3	6	6	6	5	5	5	4	6	3	6	5	5	6
	1051	53	3	6	6	5	5	6	5	5	7	3	6	4	5	5
39	1042	55	3	6	6	7	6	6	6	5	8	5	6	5	6	6
	505	56	3	1	6	7	6	6	5	7	8	3	8	6	7	6
	506	57	3	1	6	7	5	7	6	6	7	4	10	5	6	6
	1067	70	3	6	9	8	8	9	7	6	8	4	9	8	8	8
	1077	63	3	6	7	7	7	7	6	8	8	4	9	7	7	7
40	1032	61	3	6	9	8	9	8	6	5	8	3	8	8	8	7
	1030	59	3	6	7	6	6	6	6	6	7	3	7	6	6	6
	1033	58	3	6	7	7	7	6	6	5	7	4	7	6	6	6
				Total read	**64**	**64**	**64**	**64**	**64**	**63**	**60**	**63**	**63**	**63**	**64**	**64**
				Total NOT read	**0**	**0**	**0**	**0**	**0**	**1**	**4**	**1**	**1**	**1**	**0**	**0**

Table 2.- Wilcoxon signed rank test for the readings of otolith collection.

Inter-reader bias test and reader against MODAL age bias test											
	IEO Reader 1	IPIMAR Reader 2	IEO Reader 3	FREMEF Reader 4	AZTI Reader 5	CEFAS Reader 6	CEFAS Reader 7	CEFAS Reader 8	MI Reader 9	MI Reader 10	IPIMAR Reader 11
Reader 1		–	–	–	–	–	**	**	**	**	**
Reader 2	–		–	–	*	–	**	**	**	**	–
Reader 3	–	–		–	–	–	**	**	**	**	**
Reader 4	–	–	–		*	–	**	**	**	**	*
Reader 5	–	*	–	*		*	**	**	**	**	**
Reader 6	–	–	–	–	*		**	**	**	**	–
Reader 7	**	**	**	**	**	**		**	**	**	**
Reader 8	**	**	**	**	**	**	**		**	**	**
Reader 9	**	**	**	**	**	**	**	**		**	**
Reader 10	**	**	**	**	**	**	**	**	**		**
Reader 11	**	–	**	*	**	–	**	**	**	**	
Reader 12	**	*	**	**	**	–	**	**	**	**	–
MODAL age	–	–	–	–	–	–	**	**	**	**	**

–	= no sign of bias (p>0.05)
*	= possibility of bias (0.01<p<0.05)
**	= certainty of bias (p<0.01)

Table 3.- Average percent age error (**APE**), Mean coefficient of Variation (**CV**), and Index of precis obtained in the reading of otolith collection ; **n**: number of otoliths read.

	APE	mean CV	mean D	n
ALL READERS	**28.2**	**40.1**	**11.6**	**199**
READERS 3 AND 4	**6.4**	**9.0**	**6.4**	**199**
READERS 1,2,3,4 and 5	**13.1**	**16.9**	**7.6**	**199**

Figure 1.- Flow chart of activities during the Third hake otolith exchange.

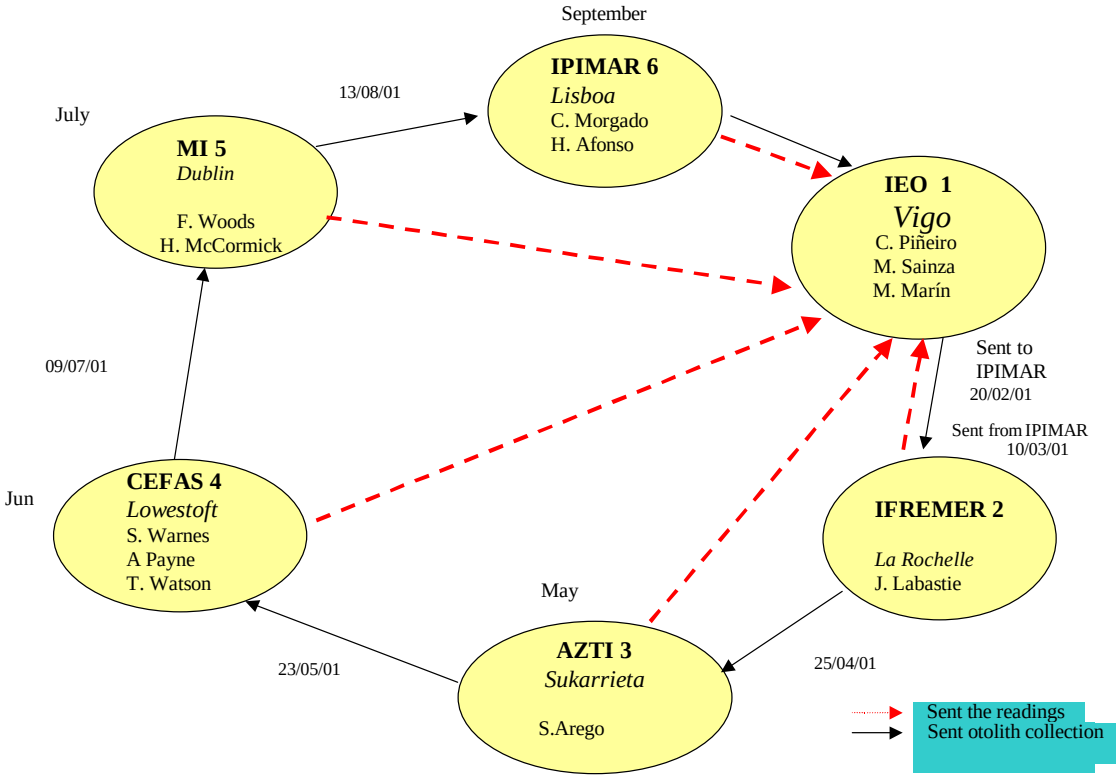
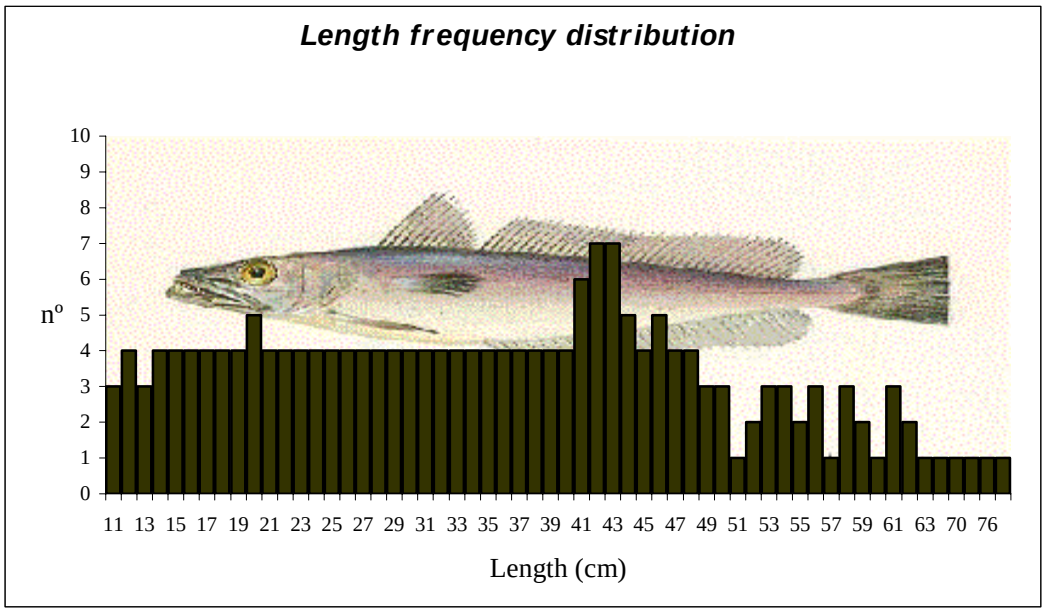


Figure 2.-Length frequency and age reading distributions of the collection sampled.



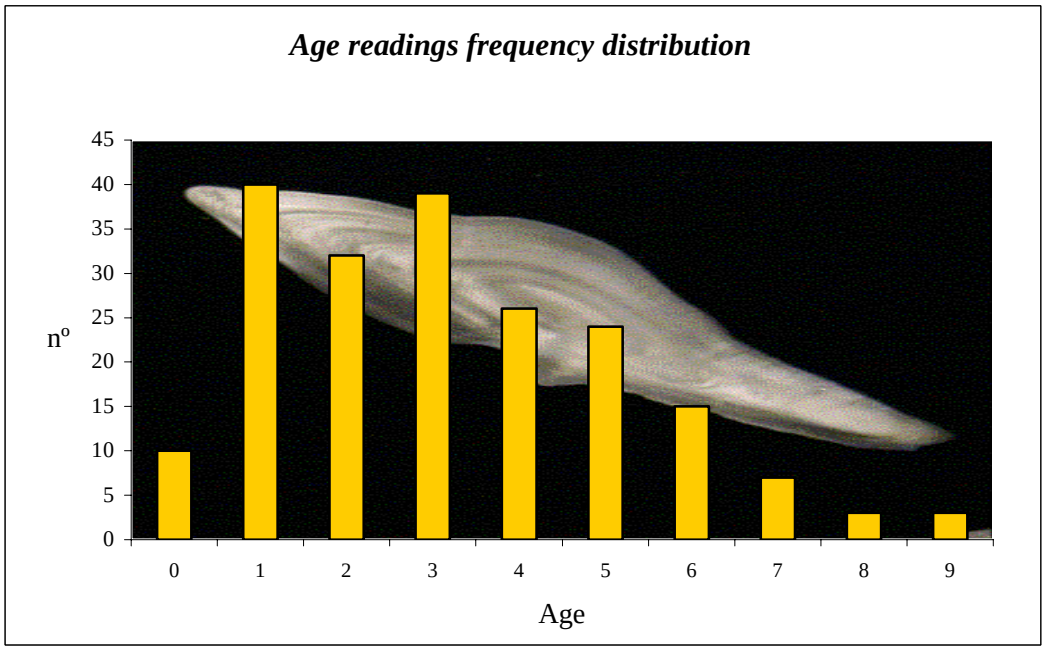
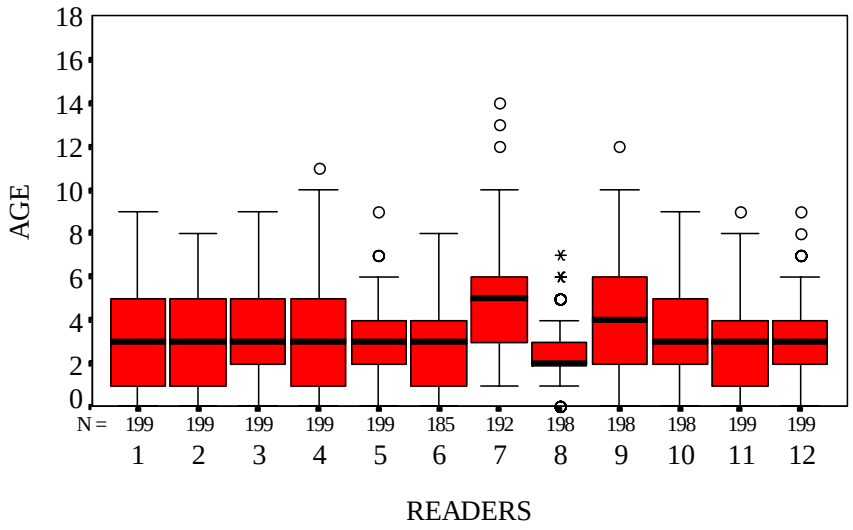


Figure 3.- Box-whisker plot of all readings (a), for first (b) and check ring (c) distances.

(a)



(b)

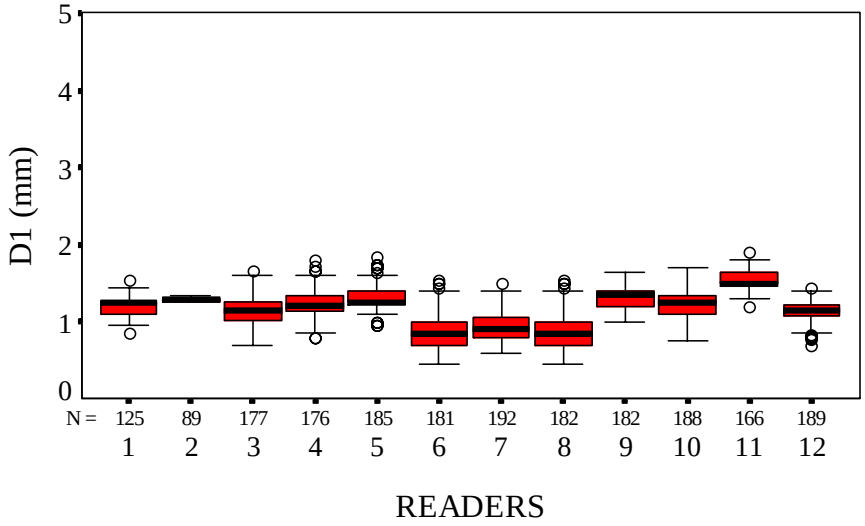


Figure 3 (cont.).- Box-whisker plot of check ring (c)

(c)

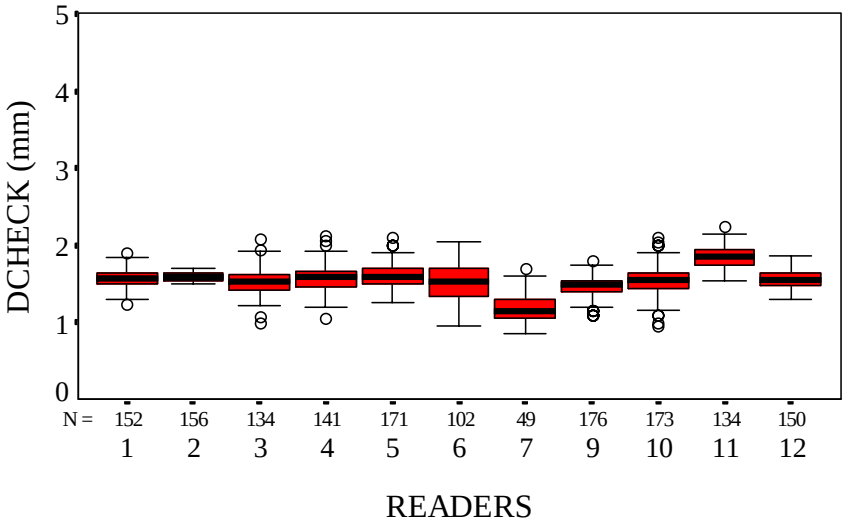
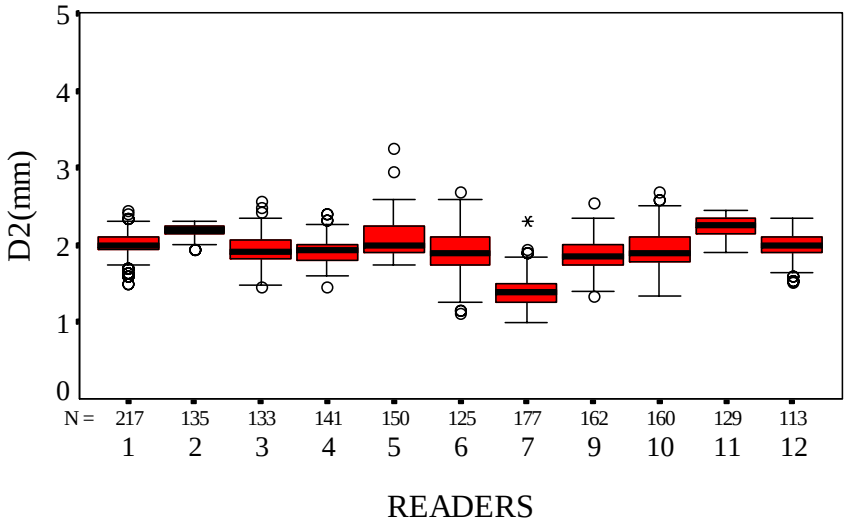


Figure 4.- Box –whisker plot for second (a) and third (b) ring distances measured for all readers.

(a)



(b)

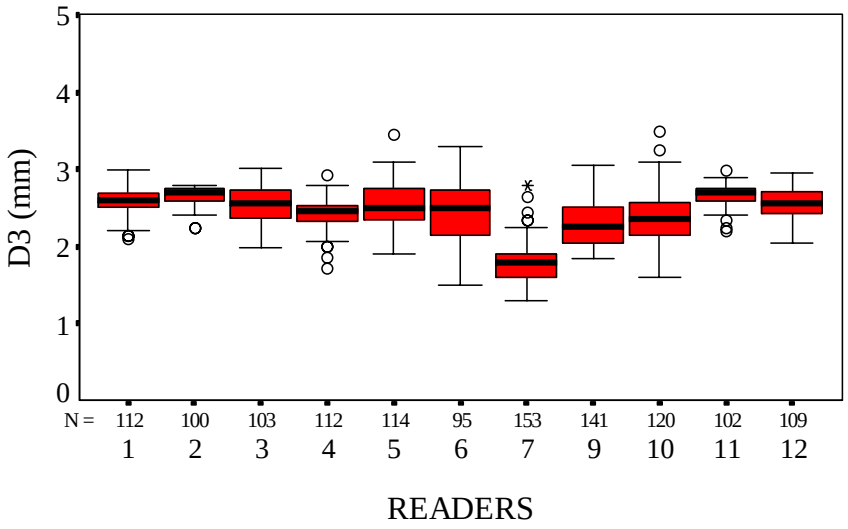


Figure 5.- Mean age recorded (±2*std) of each age reader and all readers combined against the modal age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

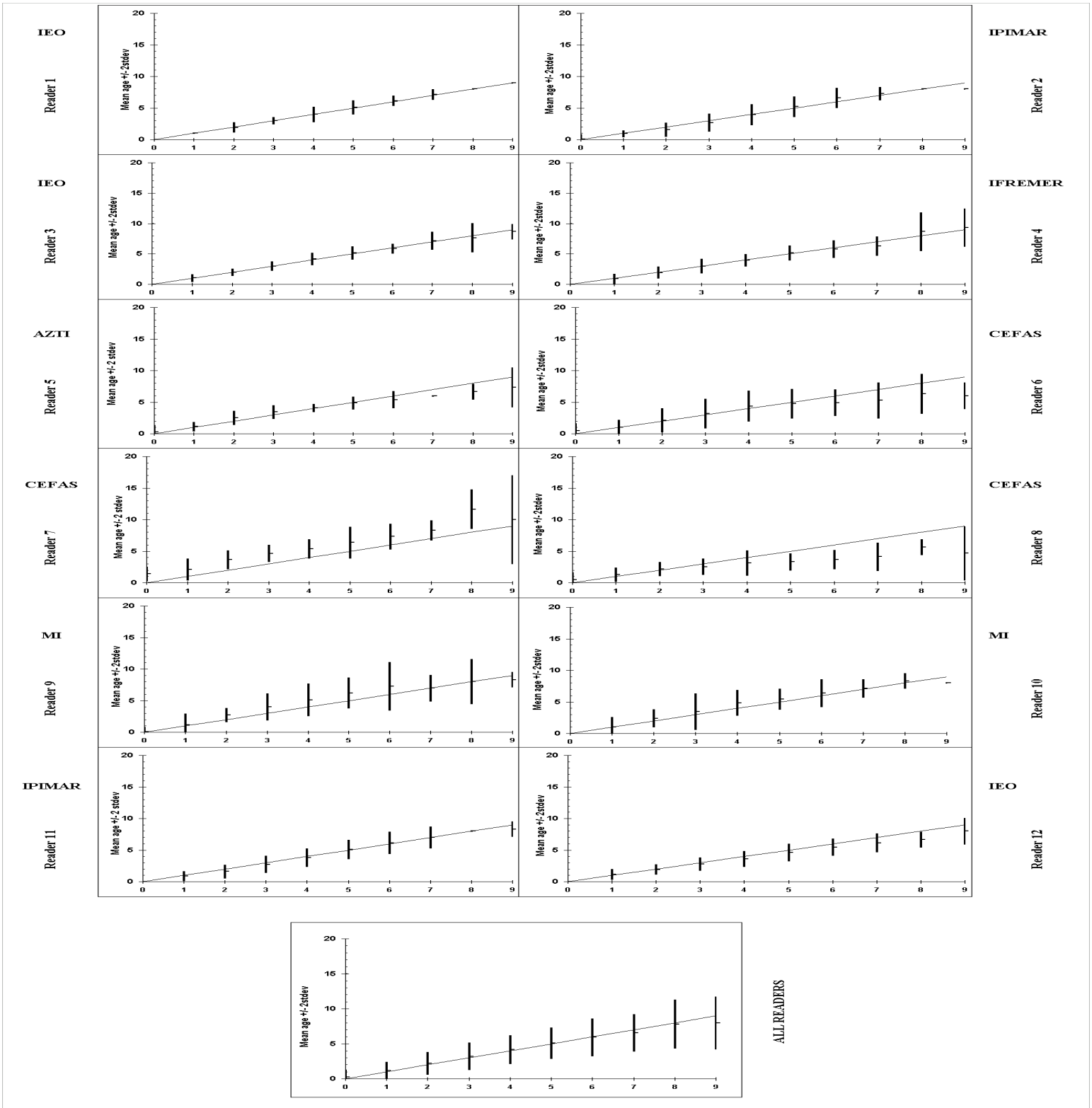
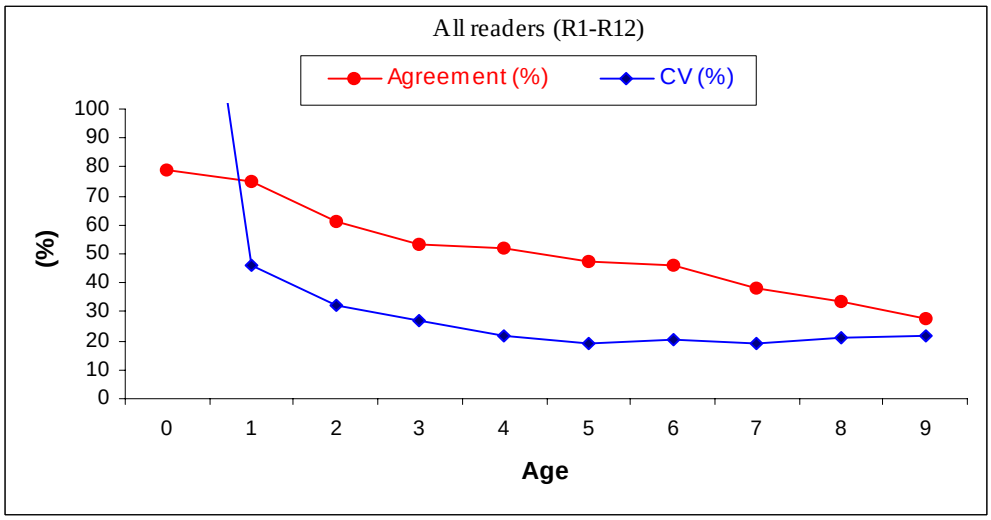
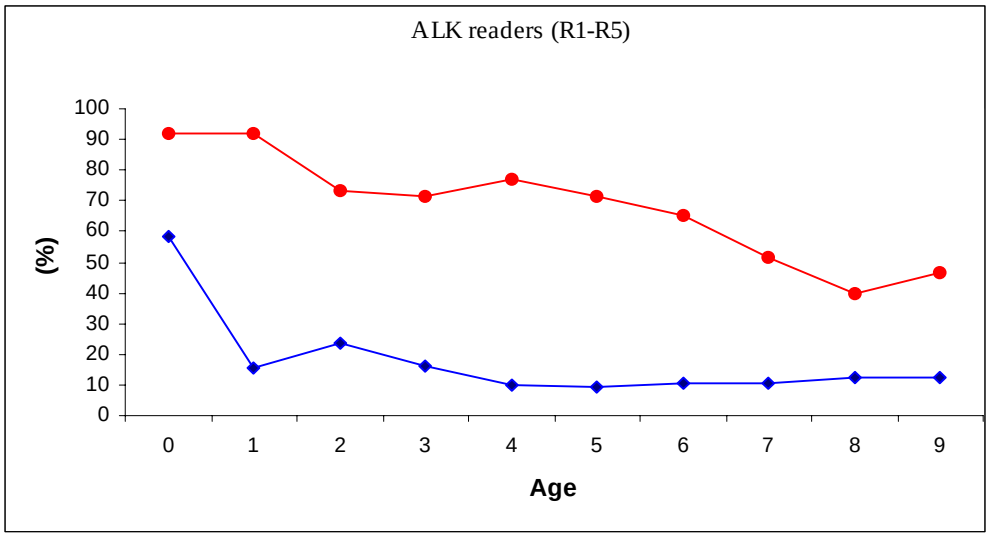


Figure 6.- The coefficient of variation (CV%) and percent of agreement (%)) from ALL readers ; experienced readers involved in ALK's (R1,R2,R3,R4,R5,) and most experienced readers (R3, R4) are plotted against MODAL age.





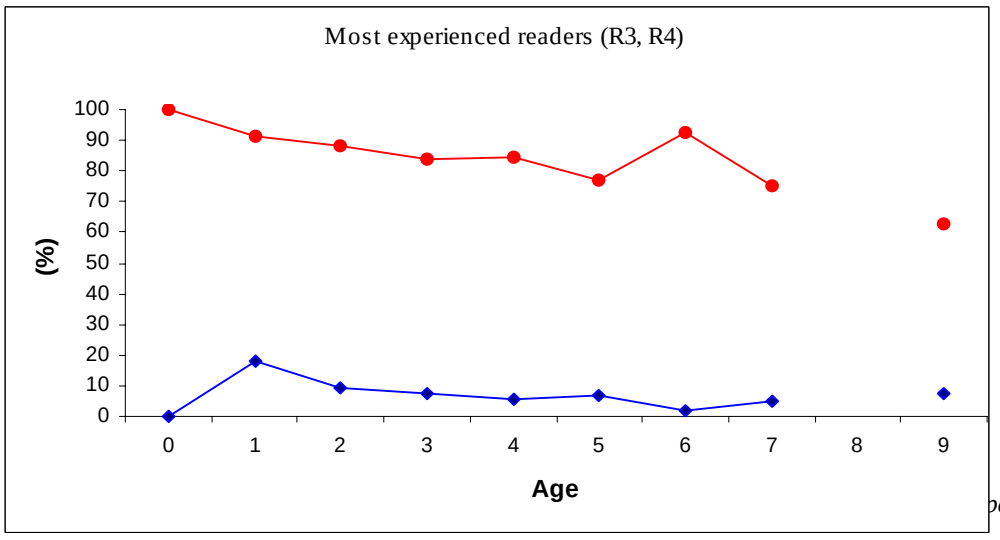


Figure 7.- Mean length at age estimated by reader.

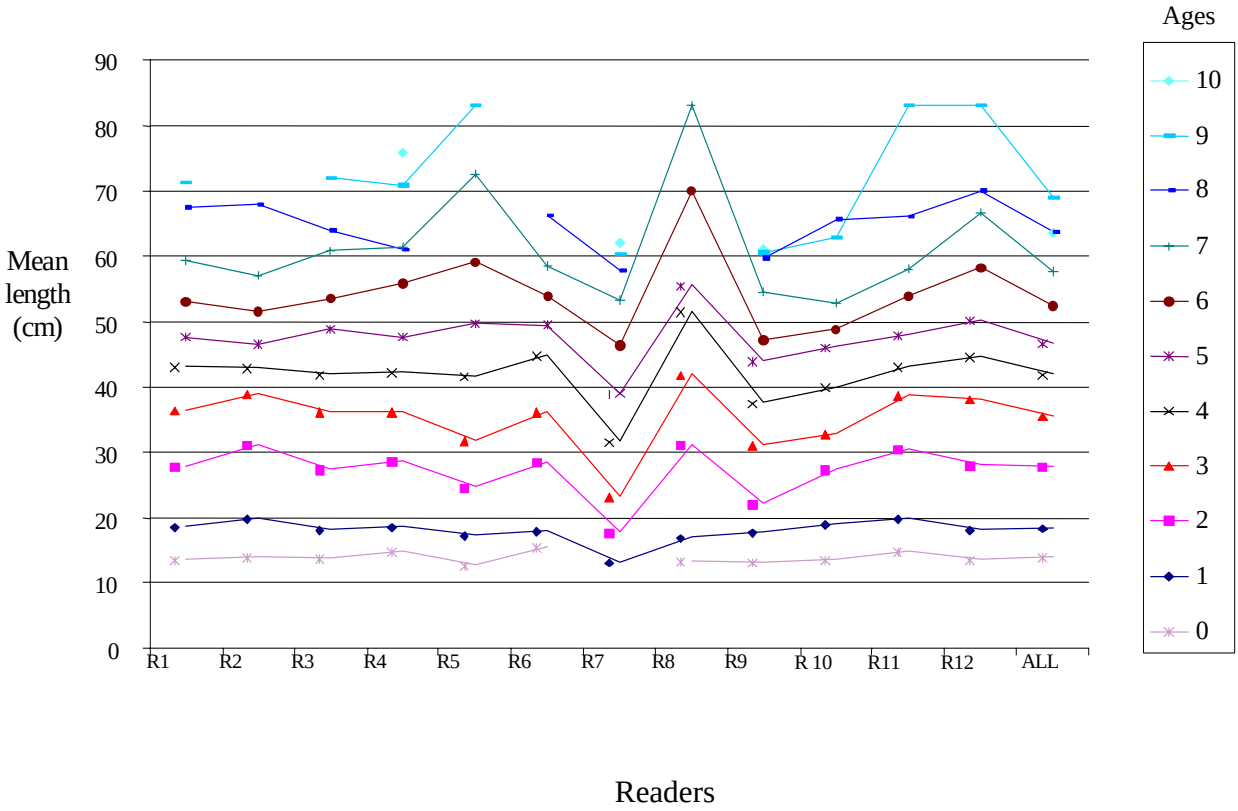


Figure 8.- Indices of Beamish and Fournier (APE), Coefficient of variation and the Index of Precision (D) observed for all readers combined, experienced readers (Involved in stock assessment: R1, R2, R3, R4, R5) and most experienced readers (R3 and R4) through all series of exchanges.

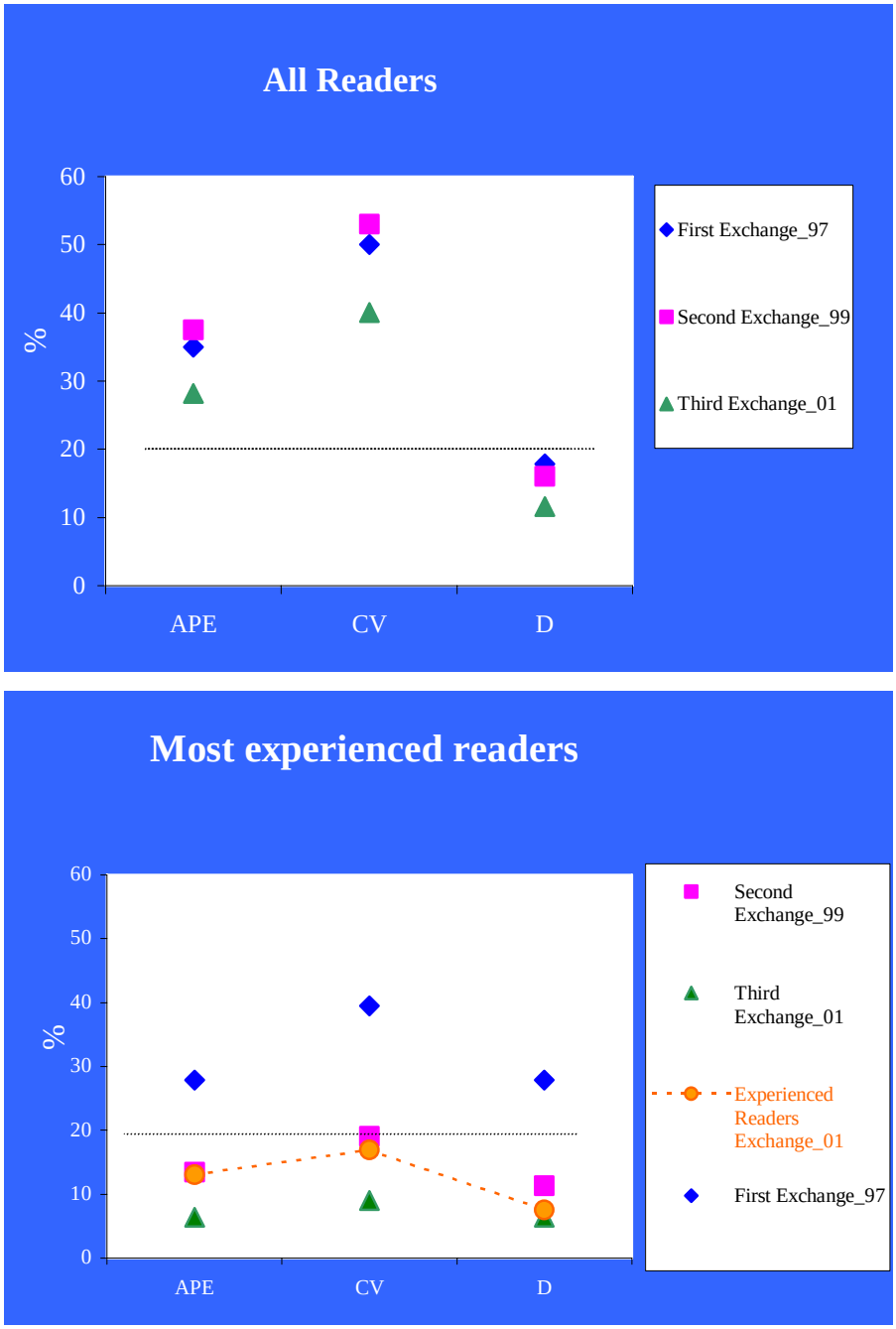


Figure 9- Dendogram obtained from the hierarchical cluster analysis.

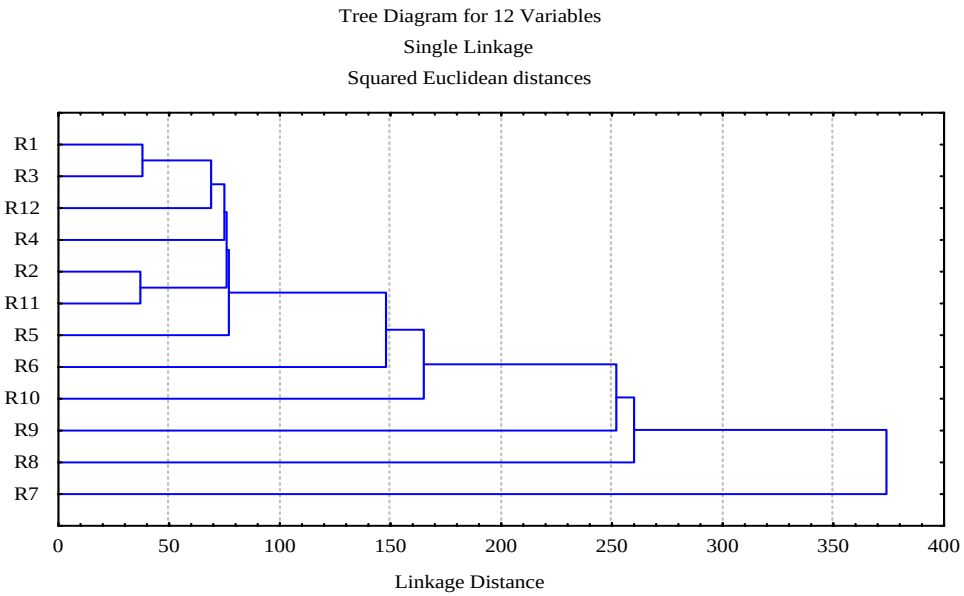
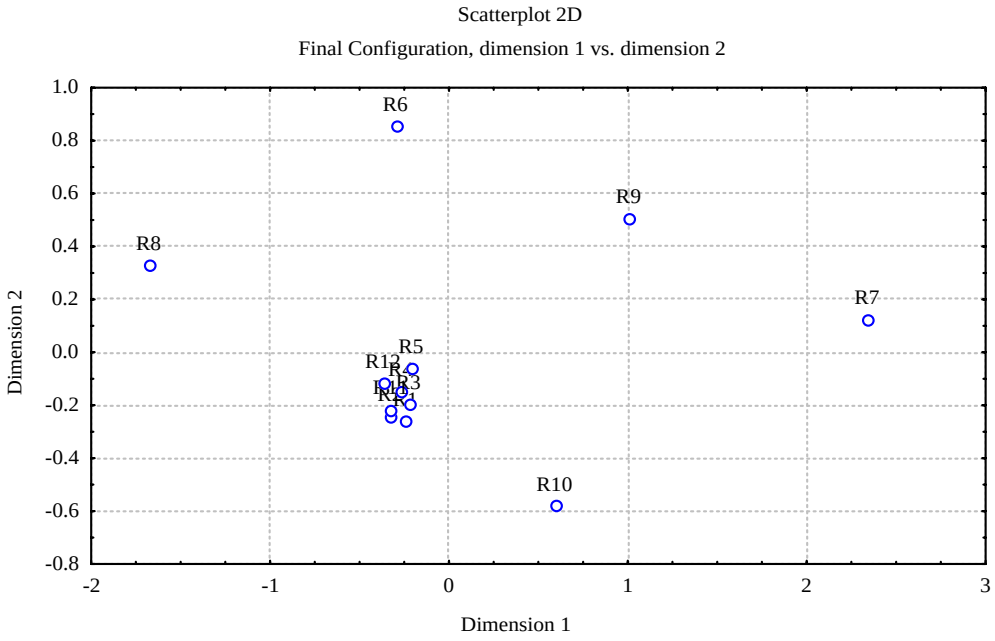


Figure 10.- MSD plots analysis.



Annex 1

PROTOCOL OF 3th EUROPEAN HAKE AGE READINGS OTOLITH EXCHANGE 2001

Dear Colleague,

According to the work plan of EU contract 99/009 (Improving Sampling of Western and Southern European Atlantic Fisheries – SAMFISH) several otolith exchange schemes will be undertaken. This protocol is concerned with European Hake exchange.

In order to continue the work initiated in 1997 and to check the precision in age reading and bias of the age readers a new hake otolith exchange will be carried out. At the start of the exchange it is very important the background of ageing this species and have some idea of the problems found so far, then please read two reports of Hake *Otolith Age Reading Workshops* conducted previously (*1997 and 1999*). They are available in the EFAN home page (*http://www.efan.no*; *Report 6-2000 and Report 7-2000*)

The exchange will be carried out following the recommendations of the EFAN Report 3-2000 on Guidelines and Tools for Age Reading Comparations, which is available in the EFAN home page*).*

A collection of 200 Hake otolith sections from two ICES areas, (100 from Sub-area VII and 100 from Division VIIIc + IXa) will be exchanged among interested institutes during 2001. The otoliths sections were prepared in Vigo (IEO) with the same technique used in the previous exchanges.

PARTICIPANTS:

- AZTI, Sukarrieta (Spain)
- CEFAS, Lowestoff (U.K.)
- IPIMAR, Lisbon (Portugal)
- IFREMER, La Rochelle (France)
- IEO, Vigo, (Spain)
- MI, Dublin, (Ireland)

Before circulating the otolith collection the following information should be taken into account for the successful completion of the exchange:

1- Otolith interpretation should be done following the standard ageing criteria already established (consult Hake Workshops Reports 1997, 1999).

2- Annual growth cycle consist in one opaque and one translucent or hyaline zone. The first of January is conventionally adopted as hake birthday.

3- Is very important to determine the position of the **First annual ring** (or growth ring) because this will be probably one of the main sources of discrepancies among readers.

4- In the samples some rings considered for age estimations should be measured in order to know whether the readers count the same rings or not. Due to time consuming, we suggest to measure only **the first three rings**, **check (Ch)** and **Rt**. The measurements are not taken from the nucleus to the outer edges of the translucent rings, because the exact point of the nucleus is often not well defined so they should be taken as follows: (see otolith drawing).

R1 (Radius of the first year's growth zone): distance between the Centrum of the otolith section and the outer edge of first translucent ring.

Dr2 (Radius distance of the second year's growth zone): distance between outer edge of **R1 and** outer edge of the second translucent ring**.**

Dch (Check distance): distance between outer edge of **R1** and outer edge of the false annual ring (Quite frequent).

Dr3 (Radius distance of the third year's growth zone): distance between outer edge of **R1** and outer edge of the third translucent ring**.**

.....

Drt (Radius of the total section): distance between the edge of **R1** and outer edge of section along the axes anterior-posterior

Edge Type:

Hyaline: 1
Opaque: 2
Unknown: 3

Reliability: (1,2,3) 1: minimum ... 3: maximum

There will be an observation cell for comments

5- An input form (Exhake_ input.xls) provides all the information on date of capture, area, etc. The readers should record the age determinations and the ring measurements as numerical format on the spreadsheet. This should be identified with name of the reader so that individual problems can be resolved at the end of the exchange

6- The otolith sections should be viewed with a binocular microscope on a black background under reflected light. The magnification used to measure rings **will be X20** and the **units** should be recorded in the spreadsheet (Exhake_ input.xls) **in mm.**

7- Digitised images (*. *tif)* for all the otoliths will be available in a CD Rom in order to help the reader interested in recording how he/she arrives at their estimated age. The CD Rom Images should accompany to the otolith collection in the running of the exchange.

The order of circulation is in the flowchart activity. Anyway, to speed up the exchange IEO institute (3 readers) will be the first one and for the reading we need whole January and part of February. The list below is ordered following the dates proposed:

Carmen Piñeiro *carmen.pineiro@vi.ieo.es*
IEO (*January- February)*
Apartado 1553
Vigo, Spain

Cristina Morgado *cmorgado@ipimar.pt*
IPIMAR (*February-Marchl*)
Avenida de Brasilia
1400 Lisboa , Portugal

Jaques Labastie *jlabas@ifremer.fr*
IFREMER (*March-April*)
BP7 17137 L'Houmeau
La Rochelle, France

Paulino Lucio *paulino@rp.azti.es*
AZTI (*Apr.-May*)
Txatxarramendi ugartea, s/n
Sukarrieta (Bizkaia) , Spain

Steve Warnes *S.Warnes@cefas.co.uk*
CEFAS (*May- Jun*)
Pakfield Road
Lowestoft, Suffolk
England, NR33 0HT

Fiona Woods *fwoods@marine.ie*
MI (*Jun.-Jul*)
Abbotstwon
Dublin 15, Ireland
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Three further items:

1. Please contact the next institution or reader on the list before dispatch in order to reduce the possibility of losing time and inform me when the otoliths have been sent.

2. Once your institute has completed the readings, please return, as soon as possible, the spreadsheet to me via e-mail with all the age reading results following the recommendations here mentioned in order to carry out successfully the age reading comparisons.

3. Is recommended to send the otolith collection with the CDRom images by special courier in order to speed up the exchange.

The Guus's spreadsheet will be used for the analysis of the exchange results (if anyone wishes to look at this spreadsheet and the accompanying guidelines they should go to www.efan.no, click on the radio button 'Guidelines' and then select 'Guidelines and Tools for Age Reading Comparisons').

If you have any queries please contact me in Vigo.
Thank you in advance for your co-operation

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telf: +34(986) 49 21 11

Hake otolith section drawing